ENZYMATIC HYDROLYSIS OF BASIC DERIVATIVES OF GLUTAMYL PEPTIDES

(Part II.)

by

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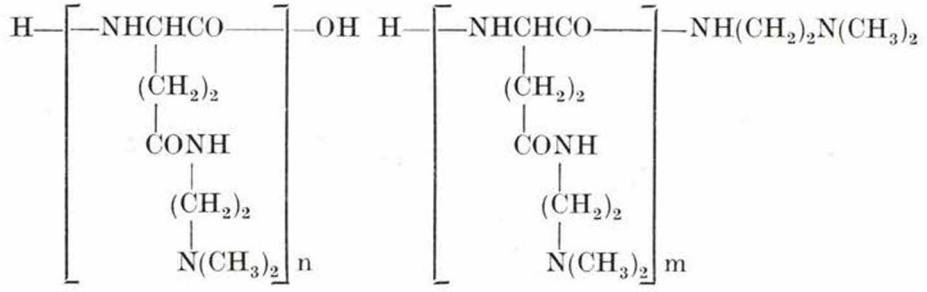
Summary

The action of different enzymes (carboxypeptidase-A, carboxypeptidase-B, trypsin and leucinaminopeptidase) was investigated on the following series of α-L-glutamylpeptide derivatives: α-diglutamyl-tri-(2-dimethylamino-ethyl)-amide, α-triglutamyl-tetra-(2-dimethylamino-ethyl)-amide, α-tetraglutamyl-penta-(2-dimethylamino-ethyl)-amide, α-penta-glutamyl-hexa-(2-dimethylamino-ethyl)-amide, α-di-[glutamyl-γ-(2-dimethylamino-ethyl)-amide] and α-tri-[glutamyl-γ-(2-dimethylamino-ethyl)-amide]. In addition a polymeric derivative; poly-N-(2-dimethylamino-ethyl)-L-glutamine was also tested. The oligomer compounds were resistant to trypsin and carboxypeptidase-A, but were split by leucinaminopeptidase. The oligomer derivatives containing free terminal carboxyl group were also split by carboxypeptidase-B, but at a slower rate than lysyl-lysine, used for comparison. The results of enzymatic digestions gave further evidence for the optical purity of the compounds investigated. The polymer was resistant to all the above enzymes.

Introduction

In our previous paper (Szókán – Kótai 1972) the action of some enzymes (chymotrypsin, trypsin, papain, leucinaminopeptidase, carboxypeptidase-A, pronase-P, subtilisin-A) on basic derivatives of glutamic acid and of some oligomer and polymer glutamylpeptides (Kótai et al. 1970 Szókán et al. 1970) was reported. All the compounds tested were resistant to trypsin, chymotrypsin, papain and carboxypeptidase-A, but were split by enzymes of bacterial origin. Oligomer derivatives containing free α-amino group were also split by leucinaminopeptidase.

Since then two series of related basic derivatives of glutamyl oligopeptides (I and II) have been synthetized in our laboratory (Szókán — Kótai 1976, Szókán 1971):



I n = 1, 2, 3 II m = 1, 2, 3, 4, 5

To get information about the behaviour of these new compounds towards enzymes, they also were submitted to enzymatic hydrolysis and were compared to natural basic substrates as well as to a polymeric derivative, consisting of similar structural units.

Materials and methods

Modified basic derivatives of glutamylpeptides were prepared as described previously:

Oligomers:

α-di-[glutamyl-γ-DMAE*-amide]	I (n = 2)	(Szókán et al. 1970)
α-tri [-glutamyl-γ-DMAE-amide]	I (n = 3)	(Szókán 1971)
α-diglutamyl-tri-DMAE-amide	II (n = 2)	(Szókán et al. 1970)
α-triglutamyl-tetra-DMAE-amide	II n(=3)	(Szókán 1971)
α-tetraglutamyl-penta-DMAE-amide	II n(=4)	(Szókán 1971)
α-pentaglutamyl-hexa-DMAE-amide	II (n = 5)	(Szókán 1971)

* In the names of peptide derivatives "DMAE" always stands for "(2-dimethylamino-ethyl)".

Polymer:

Poly-N-(2-dimethylamino-ethyl)-L-glutamine:
Poly $DMAG^{96}$ Glu^{4} (Kótai 1967)

Enzymes were obtained from the firms indicated:

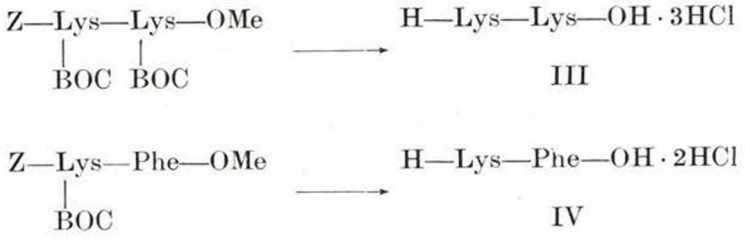
trypsin (2xcryst., lyophil.) and carboxypeptidase-A (N: 15,4%) from SERVA, Heidelberg;

leucinaminopeptidase (ex hog kidney) from Koch Light Lab. Ltd., Colnbrook;

carboxypeptidase-B from Schwarz-BioResearch, Inc., Orangeburg.

Reference substrates:

lysyl-lysine-trihydrochloride (III) and lysyl-phenylalanine-dihydrochloride (IV) were prepared in our laboratory from protected lysyl-dipeptides (Schwyzer – Rittel 1961, Marchiori et al. 1967) according to the method of Khosla (1963):



For the simultaneous removal of Z, BOC and methyl ester groups 0.5 mmole of the protected dipeptide was dissolved in 6 ml trifluoroacetic acid, 6 ml of conc. HCl was added to it and the solution was heated in a sealed tube at 40° for 4 hrs. Solvent was removed in vacuo at 20°, the residue was washed successively with ether and petrolether. The dipeptides were isolated as hydrochlorides. These dipeptide salts were chromatographically pure and were used directly for enzymatic investigations; leucinamide-hydrochloride (V) was obtained from SERVA, Heidelberg.

Method:

Digestion of the oligomers, the polymer and the reference substrates by different enzymes was followed by paper electrophoresis. Incubation mixtures containing 0.1 – 2% of substrates in suitable buffers (pH 7 for trypsin and pH 8.5 for the others) were kept at 37° in small test tubes. The reaction was initiated by the addition of the enzyme. 1 – 10 μl aliquots of the digests were withdrawn at 0,2 and 24 hours and were applied directly to Whatman No. 1 paper. Elektrophoretic patterns, obtained in a horizontal electrophoretic assembly at 1500

V, are shown in Figures 1 and 2. Digestion was indicated by the appearance of new ninhydrine positive spots, by the change in intensity of ninhydrine-colour in time, and finally by the disappearance of the starting materials.

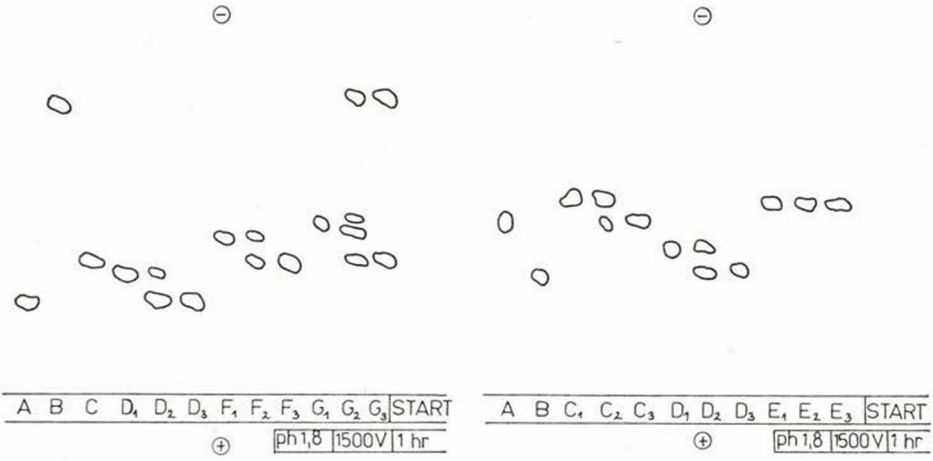


Fig. 1. Electrophoretic pattern of the digestion of basic derivatives of glutamyl peptides and leucinamide by leucinaminopeptidase.

Controls: Leucin (A), DMAE-amine (B)
γ-glutamyl-DMAE-amide (C)

Substrates: leucinamide (D) and basic derivatives of glutamyl peptides:
α-tri-glutamyl-γ-DMAE-amide (I, n = 3) (F)
α-tetraglutamyl-penta-DMAE-amide (II, n = 4) (G)*

Index 1 denotes samples applied before, index 2 samples applied after 2 hrs, index 3 samples applied after 24 – 48 hrs of digestion at 37° in the suitable buffer system (pH 8,5).

* (The members of series I and II showed similar electrophoretic patterns.)

Fig. 2. Electrophoretic patterns of basic derivatives of glutamylpeptides and lysyl-lysine digested by carboxypeptidase-B.

Controls: lysine (A)
γ-glutamyl-DMAE-amide (B)

Substrates: lysyl-lysine (C) and basic derivatives of glutamylpeptides:

α-di[glutamyl-γ-DMAE-amide] (I, n = 2) (D) and α-diglutamyltri-DMAE-amide (II, n = 2) (E).

Index 1 denotes samples applied before, index 2 samples applied after 2 hrs, index 3 samples applied after 24 or more hrs of digestion at 37° in the suitable buffer system (pH 8,5).

Results

The enzymatic sensitivities of basic derivitaves of glutamyl peptides are summarized in Table I.

Table I

Enzymatic sensitivity of basic derivatives of glutamyl peptides

Enzymes	Substrates									
	Oligomers						Polymer	Reference substrate		
	I		II							
	n=2	3	m = 2	3	4	5		III	IV	V
Trypsin	−	−	−	−	−	−	−	+	+	
Carboxypeptidase-A	−	−	−	−	−	−	−		+	
Carboxypeptidase-B	+	+	−	−	−	−	−	+		
Leucinaminopeptidase	+	+	+	+	+	+	−	+	+	+

Digestion is indicated by +, resistance by −

It can be seen that all basic derivatives of the oligoglutamyl peptides tested were resistant to trypsin and carboxypeptidase-A, but were split by leucinaminopeptidase (*Table I* and Figure 1). The oligomer derivatives of series II were split by leucinnaminopeptidase to give finally γ-glutamyl -DMAE-amide and DMAE-amine, the derivatives of series I gave only γ-glutamyl-DMAE-amide. The oligomer derivatives containing free terminal carboxyl group were also split by carboxypeptidase B to give γ-glutamyl-DMAE-amide (Table I and Figure 2). The electrophoretic patterns showed that these substances were fully digested after 1 – 2 days.

The polymer derivative was resistant to all of the enzymes investigated here.

The lysyl-dipeptides as reference substrates were very well split by the appropriate enzymes. Lysyl-lysine was split more quickly by carboxypeptidase B than the basic dipeptide of glutamic acid (Figure 2).

Discussion

Our earlier investigations (Szókán – Kótai 1972, Szókán 1971) about the resistance of some basic derivatives of glutamyl peptides to trypsin and carboxypeptidase A were further extended. The new results are consistent with our previous statement, that the presence of special, non-natural structural units in our substrates makes them resistant to these enzymes. No resistance of the same substrates was, however detected in the case of digestion with leucinaminopeptidase. This agrees with the known broad specificity of this enzyme, being able to hydrolyse a wide range of compounds containing a free α-amino group together

with an α-carboxamide or peptide bond (Hoppe-Seyler – Thierfelder 1962, Szókán – Kótai 1972). The observed resistance of the polymer to this enzyme has already been explained (Szókán – Kótai 1972) by the presence of pyroglutamyl residues at the N-terminal parts of the molecule.

The results obtained with carboxypeptidase-B have to be considered in more detail. In spite of the observed resistance to carboxypeptidase-A and trypsin, the oligomers of series I, having free terminal carboxyl group, were split by this enzyme, nevertheless at a significantly lower rate than lysyl-lysine. Carboxypeptidase-B hydrolyses the C-terminal peptide bonds of proteins and peptides only if the C-terminal amino acid is a basic one, e.g. lysine or arginine (Hoppe–Seyler – Thierfelder 1962). Its capacity of splitting our substrates containing special, non-natural structural units, indicates – compared to trypsin – a less pronounced side group specificity of this enzyme. The following two observations also support this conclusion: According to Katchalski and coworkers (1948) and to Glander – Folk (1958) polylysine was split by both trypsin and carboxypeptidase-B, but according to Seely – Benoiton (1969) poly-ε-N, ε-N-dimethyl-lysine was split only by carboxypeptidase-B, and remained resistant to trypsin.

It cannot be settled at present, however, why the polymer investigated remains still resistant to the less side-group-specific carboxypeptidase-B. It cannot be decided yet whether some effect in connection with the polymeric character, missing from the oligomers, makes an additional contribution to the observed phenomenon or else, some earlier unconsidered structural "anomaly" at the C-terminal part of the molecule is responsible for the observed behaviour. A similar explanation as this last one seemed to be plausible for the observed resistance of the polymer to leucinaminopeptidase (Szókán – Kótai 1972, Szókán 1971).

The oligomers were fully digested in all experiments. Thus the electrophoretic patterns documented not only the electrophoretic but also the optical purity of the compounds tested.

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